Integration of ecological and morphological studies: Micro-distribution of *Protaphorura*-species (Collembola: Onychiurinae) around a beech stem

JOSEF RUSEK

Rusek, J.: Integration of ecological and morphological studies: Micro-distribution of *Protaphorura*-species (Collembola: Onychiurinae) around a beech stem.
Contributions to Soil Zoology in Central Europe II. Tajovský, K., Schlaghamerský, J. & Pižl, V. (eds.): 117-120. ISB BC AS CR, v.v.i., České Budějovice, 2007. ISBN 978-80-86525-08-2

The micro-distribution of soil mesofauna was studied around a beech stem in a *Luzuleto-Fagetum* forest in the Voděradské Bučiny National Nature Reserve, ca 30 km east of Prague, Central Bohemia. The soil type was a cambisol on granite. Fifty soil samples (10 cm^2 surface area and 5 cm deep) were taken in five parallel rows at different distances below and above the stem on a gentle SW slope. Soil arthropods were extracted from each sample in a high-gradient extractor. Soil pH and bulk density were determined for each sample after animal extraction. Three *Protaphorura*-species were recorded in the samples: *P. armata*, *P. pseudovanderdrifti* and *Protaphorura* sp. nov. The eudominant *P. armata* reached highest density in samples 50 to 100 cm below and above the beech stem where *Protaphorura* sp. nov. was present only in a low frequency and at most by a single (exceptionally two) specimen per sample. *P. pseudovanderdrifti* occurred in a very low density and frequency more distant from the stem. The correlation of *Protaphorura* spp. densities with abiotic soil sample parameters was calculated and discussed. *P. armata* reached its highest density near the stem, and *Protaphorura* sp. nov. did not occur in samples with a high density of *P. armata*. These species of the genus *Protaphorura* (earlier called *Onychiurus armatus*-group), since their descriptions by Gisin in the 1950s, have been controversial, because of the seemingly variable morphological characters introduced by him. One group of collembologists therefore did not recognize them as distinct species. This study has shown that different *Protaphorura*-species are clearly separated morphologically as well as by their microhabitats around a stem in a beech forest. Integration of ecological and morphological studies could contribute to solve difficult taxonomic problems in Collembola and other soil microarthropods.

Keywords: Beech forest, acid stemflow, Collembola, *Protaphorura*-species, horizontal species micro-distribution, taxonomical consequences.

Josef Rusek, Institute of Soil Biology, Biology Centre AS CR, v.v.i., Na Sádkách 7, 370 05 České Budějovice, Czech Republic. E-mail: rusek@upb.cas.cz

Introduction

Despite the immense development of collembolan taxonomy in the last decades, there are still some urgent areas that need extensive contributions by taxonomists (Rusek, 2002). One such area that has been called into question by some authors is the status of the *Onychiurus armatus* (Tullberg, 1869) group. Starting in the 1950s, Gisin splitted the former *O. armatus* into independent, morphologically defined new species (e.g. Gisin, 1952, 1956, 1957, 1961, and others). Many authors accepted Gisin's taxonomic concept of this so-called *armatus*-group without reservation; others disregarded the splitting and considered all of Gisin's species as *O. armatus*. Bödvarsson (1970) studied the variability of the morphological characters introduced by Gisin and statistically evaluated a large number of specimens from South Sweden. He concluded that the justification for Gisin's forms (species sensu Gisin) of the *armatus*-group was not valid. The *O. armatus* forms found in his material were therefore considered as infraspecific. Pomorski (1990) performed further variability studies of some morphological characters in the *armatus*-group (belonging now to the genus *Protaphorura*) and established that only 13 of the Gisin's species studied were valid and the remaining ones were synonyms of them. The taxonomic problem around the *P. armata*-group is not as simple as stated by Pomorski (1990) and there still exists today two groups of taxonomists as in the time when Gisin started to describe his new species! This is my first contribution to the problem of the *P. armata*-species group and it deals with ecology. Further studies dealing with variability of morphological characters and with post-embryonic development are in preparation.

Distribution of Collembola in ecosystems depends on the use of different microhabitats (Rusek, 2001). Collembolan micro-distribution in soil is also correlated with chemical soil properties. Acid rain is an important factor influencing life in soil (Rusek and Marshall,

Presented at the 8th Central European Workshop on Soil Zoology, České Budějovice, April 20-22, 2005

2000). Local distribution of acid rain and other pollutants in the landscape and ecosystems depends on air turbulence levels, on snow accumulation in mountains, and on runoff gullies (Ruck and Adams, 1988; Rusek, 1993). Airborne pollutants carried by stemflow concentrate in soil near deciduous trees (Mina, 1967; Wittig, 1986; Beniamino et al., 1991). Almost 30 % of rainfall may enter the soil near the trees as stemflow in beech forests (Kazda et al., 1986). Collembola are highly sensitive to pH changes and heavy metal concentration as was shown for the infiltration zone in beech forests (Kopeszki, 1991, 1992a, b; Rusek and Marshall, 2000). A team of soil biologists and ecologists from our Institute have studied the impact of polluted rain on different groups of soil biota and soil chemistry in a protected beech forest complex at Voděradské Bučiny east of Prague (Skřivan et al., 1995). The pH distribution in 50 soil samples on the slope below and above a beech stem did not show significant differences among the samples as were reported by e.g. Kopeszki (1992b). The material from the 50 quantitative soil samples contained 2399 specimens and 54 species of Collembola. The distribution of some species of Collembola, their co-existence in the same micro-sample, as well as their micro-distribution in samples near and more distant from the tree proved useful for species preference studies. Three *Protaphorura*-species found in the samples below and above the beech stem were used for a detail micro-distribution analysis.

The goals of the present paper are:

1) To describe the micro-distribution of three *Protaphorura*-species in 50 soil samples taken at different distances above and below a beech stem;

2) To analyse and correlate the densities among the species with soil pH and soil bulk density;

3) To discuss the implications of species micro-distribution for taxonomy.

Material and methods

The investigations were carried out in the Voděradské Bučiny National Nature Reserve near Jevany u Prahy, about 30 km west of Prague, Central Bohemia, Czech Republic, 14°48' E, 49°58' N. The protected area is 406 – 500 m a.s.l. with a total annual precipitation of 635 mm and a mean annual temperature of 7.5°C. The parent bedrock is granite, and soils are mostly oligotrophic cambisols. The prevailing vegetation belongs to different beech forest communities. The investigated site was on a 30° SW slope in a *Luzuleto – Fagetum typicum* forest community. The selected beech tree was 60cm in diameter and about 100 years old and the soil was covered by beech litter on the whole study plot. Fifty soil samples were taken in five rows on the slope below and above the tree stem according to the following scheme (Fig. 1). The rows close below and above the stem were separated to sub-rows with 5 samples 0.5 m and 1m distant from the stem because of the expected stemflow range. A distance of 1 m separated the remaining rows and the samples in a row were separated by 10 cm.

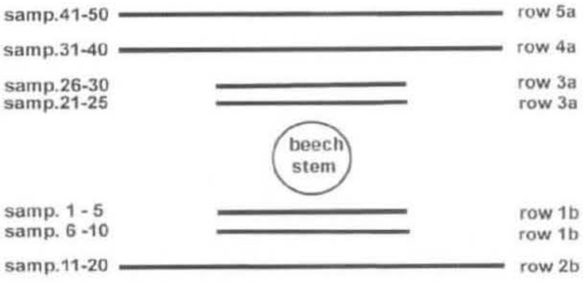

Fig. 1. Sample arrangement below (rows 1b, 2b) and above (rows 3a-5a) the beech stem. Rows 1b and 3a were divided into two parts 0.5 and 1m from the stem and each part contained 5 samples. Row 2b and 4a were 2m and row 5a 3m from the stem.

Soil samples of 10 cm² surface area and 5 cm deep were taken on April 6th 1994 by a cylindrical soil corer and transported in plastic bags to the laboratory for arthropod extraction in a high-gradient apparatus. The samples were weighed before and after soil animal extraction, and this data, plus the volume of samples, were used for soil bulk density calculation (Table 1). Dry soil samples were used for pH analysis (pH in H_2O - 1: 2.5) (Table 1). The arthropods were extracted one week into small containers with concentrated picric acid solution and transferred into 96% ethanol for conservation and further manipulation. The arthropod material was sorted into Collembola, Oribatida, other Acarina and other Arthropoda using a Technival stereomicroscope. All specimens of *Protaphorura*-species were mounted on microscopic slides for determination (Rusek, 1975). The *Protaphorura*-species were determined using a Polyvar widefield microscope with phase contrast. The statistical evaluation of *Protaphorura*-species micro-distribution in the samples and species correlation with soil bulk density and pH were performed by the Kruskal-Wallis One Way Analysis of Variance on Ranks and by the Spearman Rank Order Correlation using the statistical program SigmaStat (1997).

Results

Three *Protaphorura* species – *P. armata* (Tullberg, 1869), *P. pseudovanderdrifti* (Gisin, 1957) and an undescribed *Protaphorura* sp. nov. – were found in the 50 soil samples collected above and below the beech stem. *P. armata* was most numerous (428 specimens), followed by the new species (21 specimens), while *P. pseudovanderdrifti* was very rare (4 individuals). They represented 18.9 % of the total collembolan material. *P. armata* was missing only in four of the 50 samples and is therefore a constant member of the collembolan community. Its distribution was variable in individual

Table 1. Number of individuals of *P. armata* (Prot.arm.), *Protaphorura* sp. nov. (Prot.sp.n.), *P. pseudovanderdrifti* (Prot.pse.), soil pH, and bulk density values (bulk.dens.) in the five rows (Row No.) and individual samples (Samp.No.).

Samp.No.	Row No.	Prot.arm.	Prot.sp.n.	Prot.pse.	soil pH	bulk dens.
1	1b	12	0	0	3.36	0.13
2	1b	3	0	0	3.48	0.4
3	1b	6	0	0	3.5	0.27
4	1b	31	0	0	3.44	0.4
5	1b	13	0	0	3.4	0.19
6	1b	1	1	0	3.52	0.28
7	1b	2	1	0	3.49	0.21
8	1b	14	0	0	3.47	0.18
9	1b	24	0	0	3.31	0.4
10	1b	3	0	0	3.47	0.31
11	2b	2	0	0	3.55	0.2
12	2b	1	1	0	3.67	0.23
13	2b	0	0	0	3.68	0.27
14	2b	1	0	1	3.68	0.31
15	2b	5	0	0	3.47	0.19
16	2b	5	2	0	3.34	0.22
17	2b	2	0	0	3.44	0.16
18	2b	2	0	0	3.76	0.22
19	2b	3	0	0	3.52	0.24
20	2b	0	0	0	3.56	0.36
21	3a	38	0	0	3.43	0.28
22	3a	7	1	0	3.42	0.17
23	3a	9	1	0	3.6	0.27
24	3a	12	2	0	3.47	0.48
25	3a	6	1	0	3.5	0.2
26	3a	32	0	0	3.45	0.22
27	3a	12	0	0	3.41	0.35
28	3a	21	0	0	3.43	0.69
29	3a	28	0	0	3.51	0.18
30	4a	13	2	0	3.57	0.19
31	4a	12	3	0	3.68	0.18
32	4a	5	0	0	3.45	0.39
33	4a	8	0	0	3.61	0.24
34	4a	3	2	0	3.54	0.5
35	4a	14	0	0	3.42	0.52
36	4a	13	2	1	3.58	0.22
37	4a	6	1	0	3.61	0.15
38	4a	6	0	0	3.55	0.11
39	4a	5	2	0	3.48	0.17
40	4a	0	0	0	3.42	0.32
41	5a	4	0	0	3.53	0.33
42	5a	6	0	1	3.49	0.24
43	5a	9	0	0	3.46	0.46
44	5a	0	0	0	3.59	0.81
45	5a	9	0	1	3.45	0.5
46	5a	5	0	0	3.41	0.33
47	5a	7	0	0	3.42	0.44
48	5a	3	0	0	3.35	0.48
49	5a	2	0	0	3.36	0.36
50	5a	3	0	0	3.35	0.35

samples, ranging from zero to 38 ind/10 cm^2 (Table 1). Its highest density was reached in some samples 50 to 100 cm below and above the beech stem where *Protaphorura* sp. nov. was only in a low frequency and represented at most by a single (exceptionally two) specimen per sample. The density of *P. armata* was highest in the rows close (0.5 and 1 m) above and below the stem and lowest 2 m below it. The differences in the median values among the different rows of samples were greater than would be expected by chance; there was a statistically significant difference ($P = < 0.001$) in median density values of *P. armata* among the rows. Spearman Rank Order Correlation showed that *P. armata* tended to decrease in numbers with increasing soil pH (correlation coefficient = -0.297, $P = 0.0366$), whereas *Protaphorura* sp. nov. tended to increase in density with increasing soil pH (correlation coefficient = 0.295, $P = 0.0374$) and decreased with increasing soil bulk density (correlation coefficient = -0.284, $P = 0.0459$). There was no significant relationship between the density numbers of *P. armata* and *Protaphorura* sp. nov. *P. pseudovanderdrifti* was eliminated from the calculations due to its very low frequency.

Discussion

The project was originally designed to study the impact of polluted acid rain stemflow on the distribution of soil meso- and microfauna and on soil chemical parameters in the vicinity of a beech stem. Soil pH did not show drastic differences among the sample rows close to and more distant from the stem as was recorded in the rendzina soils near Vienna (Kopeszki, 1991, 1992a, b). Nevertheless, the occurrence of three different *Protaphorura*-species and their micro-distribution around the beech stem in our study was used to analyse their ecological behavior. The correlation analysis proved that there was a significant negative relationship between the density numbers and the micro-distribution of two *Protaphorura*-species in the small area around the stem and in the small soil samples. This means that *P. armata* and *Protaphorura* sp. nov. behaved ecologically as true species. *Protaphorura* sp. nov. could live together in the same sample with *P. armata* only in cases when they did not reach high densities. These species were positively or negatively correlated with soil pH. *P. armata* tended to decrease in numbers with increasing soil pH and was more tolerant to soil acidification, whereas the density of *Protaphorura* sp. nov. tended to increase with increasing soil pH, and was less tolerant to more acid soils. Furthermore *Protaphorura* sp. nov. tended to decrease in density with increasing soil bulk density. In the present study, *P. pseudovanderdrifti* was too poorly represented to show any relationship with soil bulk density or pH, but it was dominat in *Luzuleto-Piceetum* in the same forest complex in the Voděradské Bučiny National Nature Reserve (Rusek, 2001).

These findings are of great importance for taxonomy. We could have theoretically more than one *Protaphorura*-species in one small sample. This was documented by the occurrence of two species in the same sample in our study. With increasing sample size, e.g. to one cubic decimeter, it is possible to have three, five or more congeneric species in such large sample. A "purist" taxonomist could have a problem with the variability of morphological characters used for keying out the species. In mixtures of congeneric species the "variability" may distort the true situation and could lead to a false conclusion. For example, the soil samples of the present study would contain only one *Protaphorura*-species with variable pseudocelli number 33(4)/ 023/ 3334(5)3 and parallel or little convergent insertions of the four medial chaetae in front of the anal spines! Such wrong species interpretations very often occur in the literature.

An ecological approach to such complex taxonomic problems could help in their real analysis and interpretation. The present ecological study has supported the independent species status of the *Protaphorura- armata* group, with both two taxa living together in close proximity to each other, and the third one was dominant in other forest type in the same area. The collembolan communities in two or more adjacent zoogeographic areas could be also composed of different *Protaphorura-* species (Rusek, 2001), but we have only restricted data to document it.

Acknowledgements

This work was supported by the project No. A6066702 from the Grant Agency of the Academy of Sciences of the Czech Republic. I am much obliged to Dr. V.G. Marshall, Victoria, BC, Canada, for critical comments and English improvement of the manuscript.

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